North American Journal of Aquaculture

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1002/naaq.10086

ARTICLE

The Effects of Different Winter Feeding Regimens on Growth, Survival, and Fatty Acid Composition of Fathead Minnow and Golden Shiners

Luke A. Roy*
School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, 203 Swingle Hall, Auburn, Alabama 36849, USA

Steven D. Rawles
U.S. Department of Agriculture, Agricultural Research Service,
Harry K. Dupree Stuttgart National Aquaculture Research Center, Post Office Box 1050, 2955 Highway 130 East, Stuttgart, Arkansas 72160, USA

Anita M. Kelly and Nathan Stone
Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, Arkansas 71601, USA

Jeonghwan Park
Department of Bio-Materials and Aquaculture, Pukyong National University, 45 Yongso-Ro, Nam-gu, Busan, Arkansas 48513, South Korea

Carl D. Webster*
U.S. Department of Agriculture, Agricultural Research Service,
Harry K. Dupree Stuttgart National Aquaculture Research Center, Post Office Box 1050, 2955 Highway 130 East, Stuttgart, Arkansas 72160, USA

Abstract

Winter mortality is a common problem for Arkansas baitfish farmers that produce Fathead Minnow (FHM) *Pimephales promelas* and Golden Shiners (GS) *Notemigonus crysoleucas*. Winter feeding programs are a potential avenue to improve survival and condition and reduce weight loss of baitfish. Methods of winter feeding vary widely among producers, and currently there are no recognized best management practices. The impacts of different winter feeding regimens on FHM and GS survival, growth, and lipid storage were evaluated in temperature-controlled aquarium systems. Fathead Minnow (mean ± SD = 0.88 ± 0.04 g) or GS (0.88 ± 0.02 g) were stocked at ambient water temperature, and the temperature was reduced to 6°C (FHM) or 8°C (GS) to mimic winter conditions. Three feeding regimens were implemented (3 tanks/regimen) that included ad libitum feeding twice per week (2×/week), once per week (1×/week), or once per month (1×/month). Significant differences in weight gain (loss), condition factor (*K*), and specific growth rate were observed after 13 weeks for FHM. Fish that were fed 2×/week gained nearly 3%, while fish that were fed 1×/week or 1×/month lost weight (2.3% and 10.1%, respectively). There were no significant differences in GS final weight (0.79–0.82 g), survival (65.0–88.3%), or weight gain (−6.84% to −9.50%) among treatments after 12 weeks. The GS from the 2×/week treatment had significantly higher *K*-values than GS that were fed 1×/week or 1×/month. Fatty acid profiles of both species differed among treatments, showing a decline in saturated fatty acids from initial levels and an increase in polyunsaturated fatty acids (PUFAs) as feeding frequency decreased. Results suggest that fish may lose weight during the winter, but it does not appear to adversely affect survival, and both species alter their fatty acid compositions to optimize n-3 PUFAs during cold water temperatures.

*Corresponding authors: royluke@auburn.edu carl.webster@ars.usda.gov
Received December 20, 2018; accepted March 14, 2019

Arkansas is a leading producer of baitfish, with more than 9,700 ha devoted to production of various baitfish species, and accounts for more than 80% of the annual baitfish production in the USA. Arkansas baitfish farmers raise primarily two species, the Fathead Minnow (FHM) *Pimephales promelas* and the Golden Shiner (GS) *Notemigonus crysoleucas*, which account for the vast majority of fish produced. These baitfish are preferred for numerous sport fish species and are cultured at high densities in outdoor earthen ponds, which are subject to cold water temperatures for several months each winter. Baitfish mortality during the winter does occur and can be a serious financial issue for producers, as it reduces profitability. Bird predation, water quality issues, disease, and harsh fluctuating winter temperatures can all contribute to fish loss.

Winter feeding is often recommended to improve survival and condition and reduce weight loss in FHM and GS during the winter months; however, there is a scarcity of data on the efficacy of winter feeding programs at specific temperatures. Winter feeding practices among baitfish farmers differ and may vary depending on water temperature, feed prices, winter weather conditions, and other factors. Both FHM and GS are found in geographic regions further north than Arkansas and are accustomed to cold temperatures in their natural environment (Stone et al. 2016, 2019). Effective feeding strategies are further complicated by large pond sizes, which in some cases may exceed 25 ha (Stone et al. 2016, 2019). During the peak of the production season, baitfish farmers typically administer small amounts of feed (10 $kg{\cdot}ha^{-1}{\cdot}d^{-1}$) compared to other aquaculture industries, such as catfish, for which feeding rates commonly exceed 150 $kg{\cdot}ha^{-1}{\cdot}d^{-1}$. Most commercial farmers feed baitfish a diet that contains 28–32% protein. Farmers fertilize ponds to provide an additional source of food for baitfish, which includes zooplankton, algae, and small insects (Stone et al. 1997). Golden Shiners can acquire 40% or more of their diet from natural foods (Lochmann and Phillips 1996), and it has been reported that FHM can supplement protein with energy obtained from consumed detrital materials (Lemke and Bowen 1998). During the winter months, natural food items in the pond become scarce, and farmers provide feed much less frequently due to cold water temperatures and weather conditions. Early work by Rowan and Stone (1992, 1994) evaluated winter feeding protocols in experimental earthen ponds in Arkansas. McNulty and Stone (1997) highlighted the benefits that winter feeding conferred on pond algae blooms. Presently, there are no universally recognized best management practices for the winter feeding of baitfish, although most farmers in Arkansas try to administer feed a couple of times per month during the winter months.

After an unusually cold winter in 2012–2013, baitfish and sport fish farmers in Arkansas reported larger than customary losses of fish when they began to harvest ponds. In total, more than 2,300 ha of FHM and GS production ponds on Arkansas baitfish farms were affected (Roy et al. 2013, 2017; Stone et al. 2016). During that winter, many baitfish farmers reported losing more than 50% of their crop. Due to weather conditions and high feed prices, many farmers did not feed the fish as frequently as they had during previous winters (Roy et al. 2013).

Survival during the winter is typically lower for small fish than for larger, more mature fish (Toneys and Coble 1979; Johnson and Evans 1990; Miranda and Hubbard 1994; Sogard 1997; Schultz et al. 1998; Hurst 2007). However, if fish are fed during the winter, it appears that survival can improve. Ludwig (1996) reported that survival of FHM was high (>75%) during the winter when the fish were fed at either 0.1% or 2% of body weight (BW); moreover, fish that were fed at 2% BW were significantly larger at spring harvest. Additionally, feeding may allow fish to consume essential fatty acids (FAs) that are required for metabolic functions (e.g., immunity, membrane integrity, and growth) as well as survival.

The objective of this research was to better understand winter feeding management practices and assess their potential to improve the survival and well-being (health) of two important baitfish species during the winter, when suboptimal water temperatures are present.

METHODS

Fish and culture conditions.— Fathead Minnow were obtained from a commercial grower (I. F. Anderson Minnow Farm, Lonoke, Arkansas) and were transported to the Lonoke Fish Disease Diagnostic Laboratory (University of Arkansas at Pine Bluff) in November 2013. The fish were stocked into a 150-L holding tank connected to a larger recirculating system. Fish were held in the laboratory for approximately 1 month prior to the commencement of the experimental trial to determine that they were disease free and fully acclimated to laboratory conditions. During acclimation, fish were fed a 35% protein, 6% lipid commercial feed (Delta Western Feed, Indianola, Mississippi) as fed in a study by Roy et al. (2017). In brief, the FA content of the diet was as follows: 14:0 (1.90%), 16:0 (10.40%), 17:0 (1.29%), 18:0 (5.09%), 16:1 (2.98%), 18:1 (n-7) (2.90%), 18:1(n-9) (oleic acid; 15.20%), 20:1 (4.89%), 18:2(n-6) (linoleic acid [LA]; 10.80%), 18:3(n-3) (alpha-linolenic acid [ALA]; 8.11%), 20:3 (10.50%), 20:5(n-3) (eicosapentaenoic acid [EPA]; 6.37%), and 22:6(n-3) (docosahexaenoic acid [DHA]; 9.62%); in the preceding notation, the number to the left of the colon is the number of carbon atoms, the number to the right of the colon is the number of double bonds, and the number after the hyphen is the position of the first double bond from the methyl

end). After acclimation, FHM (mean individual weight ± SD = 0.88 ± 0.04 g) were stocked into a 908-L recirculating system equipped with an in-line water heat pump (Delta Star Model DSHP-5, 0.5 hp; Aqua-Logic, San Diego, California) for temperature control. The recirculating system was configured as nine 38-L tanks (3 replicate tanks/treatment) connected to a sump and biological filter. Each tank was stocked with 20 fish and supplied with aeration from a regenerative blower connected to air stones within the tank.

Golden Shiners were obtained from a commercial grower (Treadway Fisheries, Carlisle, Arkansas), transported to the Lonoke Fish Disease Diagnostic Laboratory in November 2014, and similarly acclimated to culture conditions as previously described. After acclimation, GS (mean individual weight ± SD = 0.875 ± 0.027 g) were stocked into a 603-L recirculating system similarly configured (nine 48-L tanks, 3 tanks/treatment, sump, biofilter, and aeration), with 20 fish/tank. Water temperature was maintained by a drop-in chiller (0.19 kW) with digital temperature controllers (TradeWind Chillers, Escondido, California).

The ambient starting temperature of the two systems was 20°C. After stocking, fish were allowed to acclimate for 1 d before temperature in each system was reduced over a 7-d period to the target (low) temperature of 7°C (McNulty et al. 2000), during which time the fish were not fed (actual water temperatures were 6°C for FHM and 8°C for GS). The temperature reduction rate of 2°C per day is typical for Arkansas during the onset of winter. Once the target temperature was reached, feeding was initiated. Flow rate was set at 2.4 L/min in each culture tank within both systems. Temperatures were monitored twice daily (morning and evening) and adjusted as needed. Dissolved oxygen was monitored daily, and total ammonia nitrogen, nitrite-nitrogen, and pH were measured weekly during both trials (Table 1). Lighting in the room, which housed the experimental recirculating systems, was set at 14 h light : 10 h dark.

Fish were offered the commercial feed ad libitum according to one of the following three feeding regimens: twice per week (2×/week), once per week (1×/week), or once per month (1×/month). After 13 weeks (FHM) or 12 weeks (GS) of feeding at low temperature, the trials were terminated to assess growth, survival, specific growth rate (SGR), and condition factor (*K*). Samples of whole fish (10 FHM; 58 GS) were collected at the start of each trial (i.e., initial fish) and were obtained from each tank at the end of each trial (10 FHM/tank; 11–17 GS/tank); the samples were frozen (−20°C) for later determination of whole-body FA composition.

Fatty acid analysis.— Whole fish were stored frozen at −20°C until analysis. Fish were weighed whole, lyophilized (Labconco Triad Model 7400040 freeze dryer; Labconco, Kansas City, Missouri), and ground in a laboratory blender (Waring, Inc., New Hartford, Connecticut). Lipid was extracted from the lyophilized tissues by using the Folch method (Folch et al. 1957). Fatty acids were saponified and derivatized to FA methyl esters (FAMEs) according to the methods of Morrison and Smith (1964). To each extracted lipid sample, 100 μL of a tridecanoic acid (C13:0) standard solution (N-13AA7-N; Nu-Chek Prep, Inc., Elysian, Minnesota) were added as the internal standard. All samples were evaporated under nitrogen to dryness.

Fatty acid methyl esters were analyzed using a Varian Model CP3800 gas chromatograph/flame ionization detector (Varian, Inc., Walnut Creek, California) equipped with a capillary column (100 m, 0.25 mm; Varian CP for FAME CP7420), and helium was used as the carrier gas. Injector port and detector temperatures were maintained at 250°C, with constant gas flow rates (hydrogen: 30 mL/min; air: 300 mL/min; makeup gas: 31.7 mL/min). Column oven temperature was initially 100°C and held for 10 min; was increased to 160°C at a rate of 15°C per minute and held for 10 min; and finally was increased to 250°C at a rate of 2.5°C per minute and held for 10 min. Sample FAMEs were identified and quantified by comparing peak retention times and area counts to those of serially diluted mixtures of FAME reference standards obtained from Nu-Chek Prep that included GLC-473 spiked with trans 15:1, trans delta 9-16:1, 23:0, and 21:0 methyl esters and tridecanoic acid methyl ester (C13:0), which served as an internal standard for quantification of FAMEs.

Statistical analysis.— Growth and survival data were analyzed using one-way ANOVA and the Student–Newman–Keuls multiple range test in SAS version 9.3 (SAS Institute, Cary, North Carolina) according to Ott (1977) to determine whether significant differences ($P \leq 0.05$) existed among treatment means of individual fish responses. Fatty acid profiles were subjected to multivariate ANOVA (MANOVA) followed by stepwise discriminant analysis (SDA) using the STEPDISC procedure in SAS. For each

TABLE 1. Mean (±SD) dissolved oxygen (DO; mg/L), temperature (°C), pH, total ammonia nitrogen (TAN; mg/L), and nitrite-nitrogen (NO_2-N; mg/L) in aquaria during winter feeding trials with Fathead Minnow held at 6°C for 13 weeks and Golden Shiners held at 8°C for 12 weeks.

Species	DO	Temperature	pH	TAN	NO_2-N
Fathead Minnow	9.81 ± 1.08	6.20 ± 0.63	8.21 ± 0.24	0.37 ± 0.13	0.13 ± 0.15
Golden Shiner	8.54 ± 0.40	8.04 ± 0.79	7.70 ± 0.13	0.15 ± 0.08	0.02 ± 0.02

taxon, SDA was used to optimize the number of FAs in the profile required to discriminate among the following fish classes: initial fish (collected just prior to the start of feeding), fish fed 1×/month, fish fed 1×/week, or fish fed 2×/week and held at 6–8°C.

The FAs and their abundances selected by SDA were then subjected to canonical discriminant analysis (CDA) using the CANDISC procedure in SAS to (1) test the null hypotheses that FA profiles did not differ among initial fish and the fish fed at different frequencies and (2) indicate the relative contribution of each FA to class discrimination (Johnson and Wichern 2002). Wilks' lambda statistic within the MANOVA of CANDISC was used as the determinant of whether FA profiles differed among fish classes. A plot of the first two canonical variates (Can1 and Can2) was used to visually assess differences in FA profiles among fish treatments. Pooled within-treatment standardized canonical coefficients for each canonical variate were used to indicate the degree of correlation between FA and the canonical variate that maximally separated fish classes. The canonical coefficients for each FA included in the model were then ordered to determine trends in composition among the four fish classes within each species (Hair et al. 2005). The above procedure was repeated after excluding initial fish profiles in order to further discriminate among fed fish only.

Univariate ANOVA and the Tukey–Kramer multiple comparison technique were also employed to further investigate differences among fish classes for individual FAs that were found to be significantly discriminatory in the SDA. Fatty acid abundances were log transformed prior to all statistical analyses. A *P*-value less than or equal to 0.05 was considered significant for statistical comparisons.

RESULTS

Fathead Minnow

There were no significant differences in final weight ($P = 0.169$) and survival ($P = 0.691$) of FHM after culture for 13 weeks at 6°C (Table 2). Significant differences were observed in percent weight gain ($P < 0.001$), K ($P = 0.003$), and SGR ($P < 0.001$). Fathead Minnow that were fed 2×/week gained about 3% of initial BW when fed at low temperature, while fish in the other feeding treatments lost weight. Whole-body lipid levels in FHM that were fed 1×/month (7.11%) and 1×/week (7.22%) were significantly ($P = 0.023$) less than those in fish that were fed 2×/week (9.75%) as well as those in initial fish (10.57%).

Individual FAs as well as some classes of FA differed significantly among initial and post-winter-fed FHM (Table 3). With the exception of 18:0 and 22:0, saturates tended to be higher in initial fish, whereas most monoenes decreased from initial fish concentrations as feeding frequency decreased. The exceptions included 24:1, which increased as feeding frequency decreased, and cis-vaccenic acid (18:1[n-7]), which was highest in fish fed 2×/week (14.9%), nearly equal in initial fish (11.9%) and fish fed 1×/week (11.3%), and lowest in fish fed 1×/month (7.8%).

Polyunsaturated fatty acids (PUFAs) and n-3 FAs increased in concentration from initial FHM levels of 10.4% and 5.1%, respectively, to a high of 27% and 15.6%, respectively, as feeding frequency decreased to 1×/month. However, n-6 FAs were higher but equally so among fed-fish treatments (16.8–18.4%) compared to initial fish (11.7%). Hence, the ratio of n-6/n-3 FAs decreased from 2.4:1 in initial fish to a low of 1.1:1 as feeding frequency decreased to 1×/month.

Concentrations of LA (18:2[n-6]) were highest in FHM fed 2×/week (10.4%) and decreased by nearly half (5.5%) at the lowest feeding frequency (1×/month), whereas levels of LA in initial fish were intermediate (6.5%) to those in fish fed 1×/week (7.9%) and 1×/month. Levels of ALA (18:3[n-3]) were 0.10–0.36% and not statistically different among fish classes. Concentrations of 18:3(n-6) were equal to or higher than levels among initial fish, fish fed 2×/week, and fish fed 1×/week when compared to fish that were fed only 1×/month. Whole-body levels of the long-chain PUFAs arachidonic acid (ARA), EPA,

TABLE 2. Initial weight (W_i; g), final weight (W_f; g), survival (%), weight gain (%), Fulton's condition factor (K), and specific growth rate (SGR) of Fathead Minnow that were offered one of three feeding regimes for 13 weeks at 6°C. Values are means ± SD. Values with different letters are significantly different ($P \leq 0.05$).

Treatment[a]	W_i	W_f	Survival	Weight gain[b]	K[c]	SGR[d]
2×/week	0.84 ± 0.02	0.87 ± 0.03	86.7 ± 7.6	2.95 ± 1.39 z	0.74 ± 0.01 z	0.031 ± 0.020z
1×/week	0.90 ± 0.06	0.88 ± 0.05	88.3 ± 7.6	−2.30 ± 2.35 y	0.70 ± 0.01 y	−0.025 ± 0.030 y
1×/month	0.89 ± 0.05	0.80 ± 0.05	81.7 ± 12.6	−10.08 ± 2.05 x	0.66 ± 0.02 x	−0.110 ± 0.030 x
P	0.273	0.169	0.691	<0.001	0.003	<0.001

[a]Treatments are ad libitum feeding once per month (1×/month), once per week (1×/week), or twice per week (2×/week).
[b]Weight gain (% increase in W_i) = $(W_f - W_i)/W_i \times 100$.
[c]$K = 100 \times (W_f/L^3)$, where L = fish length.
[d]SGR = $(\log_e W_f - \log_e W_i \times 100)/t$, where t = days of culture.

TABLE 3. Whole-body fatty acid composition (%) of initial and fed Fathead Minnow that were held at 6°C for 13 weeks. Values are least-squares means of three replicates and the pooled SE (SE_p). Within a row, means with different letters are significantly different ($P \leq 0.05$). Treatments are defined in Table 2.

Fatty acid[a]	Initial	2×/week	1×/week	1×/month	SE_p	$Pr > F$
14:0	2.06 z	1.03 y	0.64 x	0.46 x	0.07	<0.001
15:0	1.18 z	0.70 y	0.75 y	0.81 y	0.03	<0.001
16:0	32.63 z	27.55 y	27.60 y	25.38 y	0.70	<0.001
17:0	2.63 z	1.64 w	1.84 x	2.12 y	0.05	<0.001
18:0	5.61 zy	5.08 y	6.03 z	5.92 z	0.18	0.004
19:0	0.85 z	0.00 y	0.00 y	0.00 y	0.06	<0.001
20:0	0.79 z	0.78 z	0.08 y	0.00 y	0.04	<0.001
22:0	0.56	0.00	0.75	0.00	0.41	0.238
Sum of saturates	45.76 z	36.79 y	36.94 y	34.69 y	0.75	<0.001
14:1	1.65 z	0.55 y	0.23 x	0.10 x	0.07	<0.001
16:1	9.15 z	6.48 y	4.74 x	4.02 x	0.35	<0.001
18:1(n-7) (VAC)	11.93 zy	14.93 z	11.32 zy	7.83 y	1.10	0.027
18:1(n-9)	7.23 z	3.50 zy	3.39 y	5.91 zy	1.21	0.034
20:1	1.26 zy	1.46 z	1.70 z	0.87 y	0.18	0.006
22:1	0.43	0.70	0.39	0.00	0.26	0.162
24:1	2.25 w	2.96 x	4.61 y	6.32 z	0.27	<0.001
Sum of monoenes	33.86 z	30.57 y	26.37 x	25.05 x	0.60	<0.001
18:2(n-6) (LA)	6.45 x	10.37 z	7.87 y	5.48 w	0.19	<0.001
18:3(n-3) (ALA)	0.10	0.36	0.26	0.28	0.26	0.959
18:3(n-6) (GLA)	1.83 z	1.40 z	1.28 z	0.55 y	0.19	<0.001
20:2	1.26 y	1.92 zy	2.51 z	2.48 z	0.18	<0.002
20:3 (all)	1.32 y	2.29 z	2.39 zy	3.20 z	0.21	<0.001
20:4 (ARA)	2.16 y	4.72 z	6.05 z	8.32 z	0.44	<0.001
20:5(n-3) (EPA)	1.34 w	1.72 x	2.61 y	3.53 z	0.12	<0.001
22:5(n-3) (DPA)	0.29 y	1.71 z	0.22 y	0.84 y	0.33	0.011
22:6(n-3) (DHA)	2.10 w	3.77 x	5.67 y	7.76 z	0.30	<0.001
PUFAs	10.40 x	17.90 y	20.99 y	26.98 z	0.95	<0.001
Σn-3	5.14 x	9.86 y	11.15 y	15.62 z	0.73	<0.001
Σn-6	11.71 y	18.41 z	17.71 z	16.84 z	0.56	<0.001
n-6/n-3	2.40 z	1.89 y	1.64 y	1.11 x	0.14	<0.001

[a]Fatty acid classes and abbreviations are as follows: cis-vaccenic acid (VAC); linoleic acid (LA); alpha-linolenic acid (ALA); gamma-linolenic acid (GLA); arachidonic acid (ARA); eicosapentaenoic acid (EPA); docosapentaenoic acid (DPA); docosahexaenoic acid (DHA); polyunsaturated fatty acids (PUFAs); sum of n-3 fatty acids (Σn-3); sum of n-6 fatty acids (Σn-6); and ratio of Σn-6 to Σn-3 (n-6/n-3).

docosapentaenoic acid, and DHA generally tripled in FHM as feeding frequency decreased to 1×/month.

Multivariate ANOVA confirmed that FA profiles in initial FHM could be distinguished from those of experimental fish (Wilks' lambda: $F = 47.6$; df = 27, 70.74; $P < 0.001$) based on the abundances of nine (14:1, 15:0, 17:0, 18:0, 18:2, 20:0, 20:5, 24:1, and 22:6) of the 24 measured FAs (Table 4). As illustrated in Figure 1 (upper panel), Can1 (y-axis) maximally separated initial fish FA profiles from those of the experimental fish, whereas Can2 (x-axis) maximally discriminated among FHM fed at different frequencies. The first canonical variate accounted for 64% of the variance, while Can2 accounted for an additional 31% of the variance, resulting in a cumulative variance of 96% (Table 4). The nine discriminating FAs added weight to the previously noted trends in FA classes: that is, higher saturates and lower PUFAs were found in initial fish compared to fed fish.

When FA loading scores on Can1 (Table 4) are ordered from most positive to most negative (loading scores in parentheses), levels of 24:1 (4.28), 17:0 (0.94), 18:2 (−0.86), and 20:5 (−4.73) appeared to be most influential among the nine in distinguishing initial FHM from experimental fish. As noted in Table 3, levels of 24:1 were particularly low in the initial fish compared to experimental fish, whereas levels of 17:0 were greatest in the initial FHM. Additionally, levels of LA (18:2[n-6]) were highest in the more frequently fed (1×/week and 2×/week) fish, while levels of EPA (as well as DHA) were much higher in experimental FHM compared to initial FHM. When

TABLE 4. Class means and fatty acid loading scores of the first two canonical variates (Can1 and Can2) for initial versus fed Fathead Minnow held at 6°C for 13 weeks and Golden Shiners held at 8°C for 12 weeks. Treatments are defined in Table 2. Fatty acid abbreviations are defined in Table 3.

Treatment, fatty acid, or statistic	Fathead Minnow		Golden Shiner	
	Can1	Can2	Can1	Can2
Class means[a]				
Initial	10.88	−1.64	9.11	−0.30
1×/month	−1.07	6.17	−1.70	1.68
1×/week	−4.62	1.69	−3.36	0.10
2×/week	−5.20	−6.21	−4.05	−1.48
Fatty acid loading scores				
14:0			−0.46	−1.27
14:1	0.80	0.19	1.25	0.52
15:0	0.49	2.43		
17:0	0.94	−0.72		
18:0	0.46	0.47		
18:2	−0.86	−0.70		
20:0	0.62	−0.82		
20:5 (EPA)	−4.73	−0.28		
24:1	4.28	−0.58		
22:6 (DHA)	−0.02	1.54		
Eigenvalue	47.24	23.11	42.59	1.92
Variance	0.64	0.31	0.96	0.04
Cumulative variance	0.64	0.95	0.96	1.00
R^2	0.98	0.96	0.98	0.66

[a]Class means locate the center of each group of fatty acid profiles in Figure 1. Loading scores are pooled within-class standardized canonical coefficients; scores with the largest absolute values correspond to fatty acids with the greatest discriminatory ability.

FA loading scores on Can2 (Table 4) are ordered from most positive to most negative, levels of 15:0 (2.43), 22:6 (1.54), 24:1 (−0.58), 18:2 (−0.70), 17:0 (−0.72), and 20:0 (−0.82) were most discriminatory in separating among fed groups of FHM.

To further investigate FA profiles of experimental FHM, initial fish profiles were sequestered, and only experimental fish FA profiles were subjected to an additional iteration of SDA/CDA. Four FAs—17:0, 20:0, 18.2, and 24:1—were sufficient to discriminate (Wilks' lambda: $F = 36.4$; df = 8, 42; $P < 0.001$) among FHM from the three feeding regimens (Table 5). The first canonical variate accounted for 87% of the variance and maximally separated FHM that were fed the least frequently (1×/month) from those fed the most frequently (2×/week; Figure 2). Fatty acid loading scores on Can1 suggested that 17:0 (4.95) and 18:2 (−12.25) levels were highly discriminatory, which was confirmed by treatment differences seen in the univariate ANOVA (Table 3). Specifically,

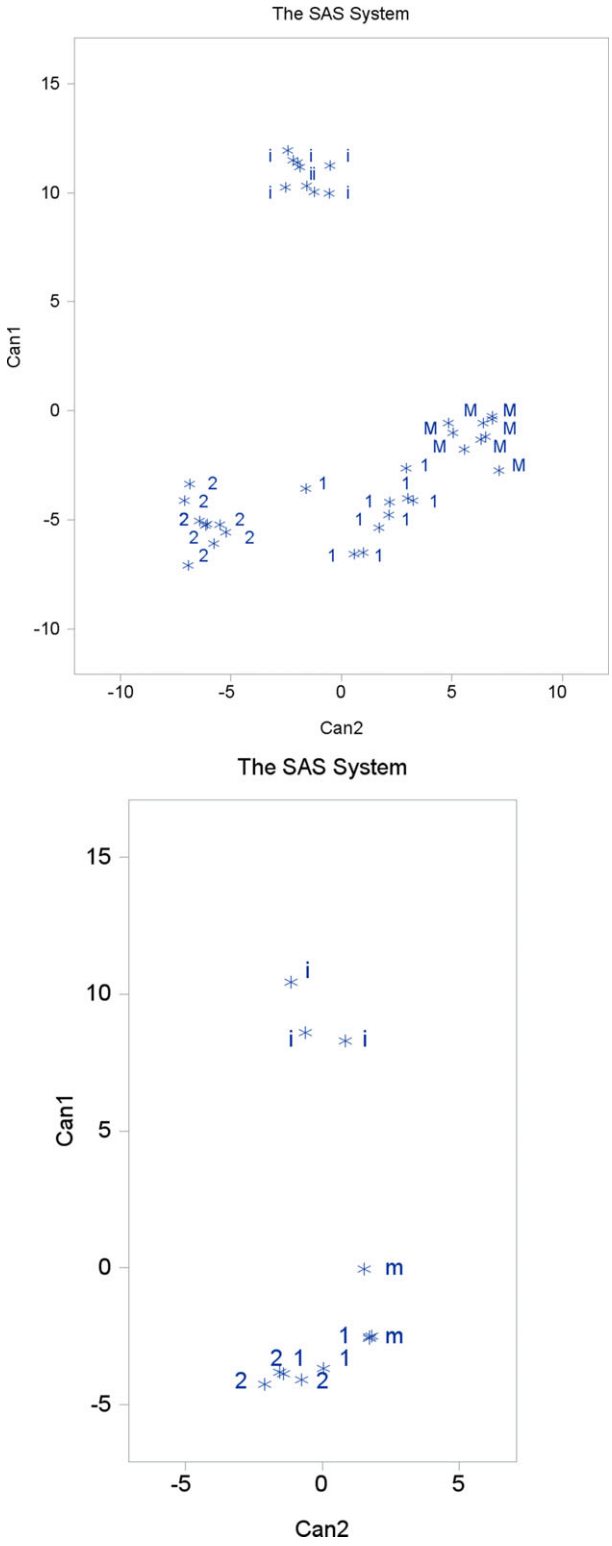

FIGURE 1. Scatter plots of the first two canonical variates (Can1 and Can2) from the analysis of fatty acid profiles in initial and fed Fathead Minnow (upper panel) and Golden Shiners (lower panel) held for 13 or 12 weeks at 6°C or 8°C, respectively. Symbols are for initial fish (i), fish fed once per month (M), fish fed once per week (1), or fish fed twice per week (2).

TABLE 5. Class means and fatty acid loading scores of the first two canonical variates (Can1 and Can2) for fed Fathead Minnow held at 6°C for 13 weeks. Treatments are defined in Table 2.

	Fathead Minnow	
Treatment, fatty acid, or statistic	Can1	Can2
Class means[a]		
1×/month	4.77	1.04
1×/week	−0.05	−2.11
2×/week	−4.72	1.07
Fatty acid loading scores		
17:0	4.95	13.96
20:0	−1.06	11.27
18:2	−12.25	−9.91
24:1	1.93	−2.26
Eigenvalue	16.91	2.51
Variance	0.87	0.13
Cumulative variance	0.87	1.00
R^2	0.94	0.72

[a]Class means locate the center of each group of fatty acid profiles in Figure 2. Loading scores are pooled within-class standardized canonical coefficients; scores with the largest absolute values correspond to fatty acids with the greatest discriminatory ability.

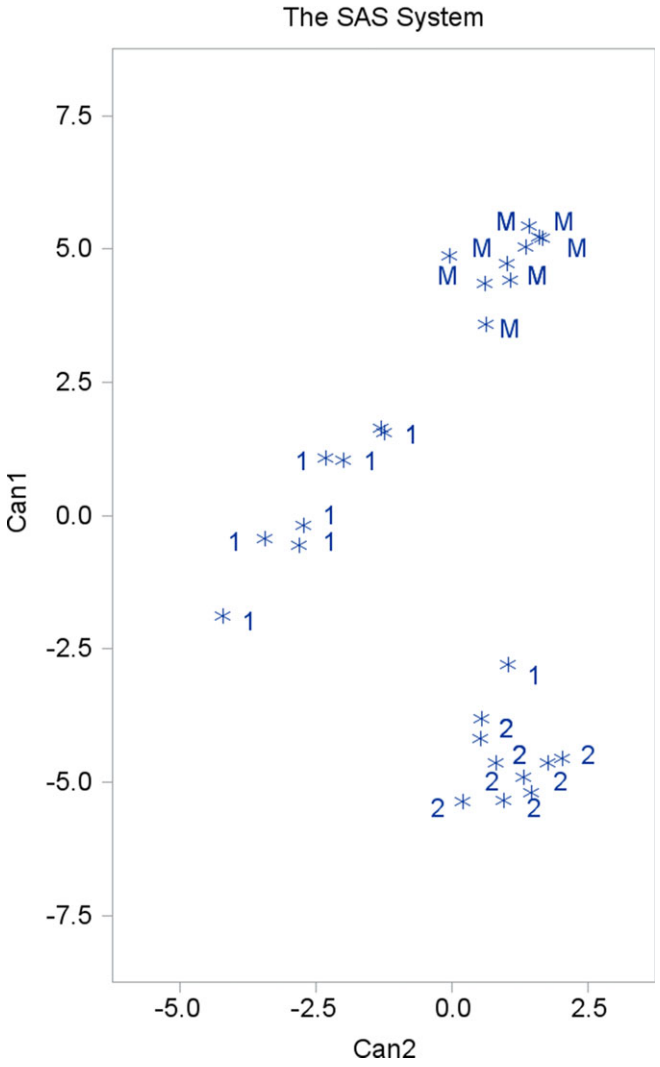

FIGURE 2. Scatter plot of the first two canonical variates (Can1 and Can2) from the analysis of fatty acid profiles in Fathead Minnow fed once per month (M), once per week (1), or twice per week (2).

17:0 levels increased while 18:2 levels decreased as feeding frequency decreased from 2×/week to 1×/month. The second canonical variate accounted for an additional 13% of the variance among FA profiles of fed FHM (Table 5) and maximally separated fish that were fed 1×/week from those fed either 2×/week or 1×/month (Figure 2). Based on FA loading scores on Can2, levels of 17:0 and 18:2 were again most discriminatory (13.96 and −9.91, respectively); levels of both FAs were distinctly intermediate in fish that were fed 1×/week compared to levels in fish fed more frequently or less frequently (Table 3).

Golden Shiner

After 12 weeks, there were no significant differences in GS final weight (0.79–0.82 g; $P = 0.804$), survival (65.0–88.3%; $P = 0.088$), or percent weight gain (−6.84% to −9.50%) among feeding regimens (Table 6). The *K*-values of fish that were fed 2×/week were significantly higher than those of fish from the other two winter feeding regimens. Whole-body lipid levels in GS that were fed 1×/month (3.01%) and 1×/week (3.24%) were significantly ($P = 0.049$) less than those in initial fish (6.23%). Lipid levels in fish that were fed 2×/week (4.75%) were numerically intermediate to (but not different from) lipid levels in fish fed more or less often and were not different from levels in the initial fish.

Individual FAs as well as some classes of FA differed among initial and experimental GS (Table 7). Levels of 14:0 ($P = 0.003$) and 15:0 ($P = 0.002$) decreased in fed GS from initial levels, while 18:0 ($P = 0.005$) increased in fed fish (6.5–7.8%) compared to initial fish (4.9%). On the other hand, the sum of saturated FAs (SFAs) did not differ significantly ($P = 0.445$) and ranged from 29.9% to 32.8% among all fish classes. The sum of monounsaturated FAs significantly decreased ($P = 0.003$) from initial fish levels (39.9%) to 27.5% in fish that were fed only 1×/month. Among the monoenes, as feeding frequency decreased, levels of 14:1 ($P < 0.001$), 16:1 ($P < 0.001$), and 18:1(n-7) ($P = 0.081$) declined or showed a tendency to decline from initial GS levels. The sum of PUFAs was higher in fed GS (33.3–38.9%) compared to initial GS (25%) but did not differ among classes of fed GS. Similarly, total n-3 FAs were higher but similar among classes of fed GS (15.3–20.6%) compared to initial fish (9%). Total n-6 FAs (15.6–17.8%) did not differ significantly ($P = 0.671$) among fish classes. Ratios of n-6/n-3 FAs,

TABLE 6. Initial weight (W_i; g), final weight (W_f; g), survival (%), weight gain (%), Fulton's condition factor (K), and specific growth rate (SGR) of Golden Shiners that were offered one of three feeding regimes for 12 weeks at 8°C. Values are means ± SD. Values with different letters are significantly different ($P \leq 0.05$). Treatments and response variables are defined in Table 2.

Treatment	W_i	W_f	Survival	Weight gain	K	SGR
2×/week	0.88 ± 0.04	0.82 ± 0.09	88.3 ± 5.77	−6.84 ± 7.32	0.59 ± 0.02 z	−0.085 ± 0.094
1×/week	0.87 ± 0.03	0.79 ± 0.01	80.0 ± 0.00	−9.50 ± 2.93	0.55 ± 0.02 x	−0.116 ± 0.034
1×/month	0.88 ± 0.01	0.81 ± 0.04	65.0 ± 17.3	−8.28 ± 6.00	0.53 ± 0.01 x	−0.102 ± 0.076
P	0.939	0.804	0.088	0.853	0.020	0.870

however, decreased from 1.8:1 in initial GS to 0.9:1 in fish that were fed 1×/month. For most cases in which individual FA abundances were significantly different between initial GS and experimental GS, there was either significant statistical overlap or no significant difference among the fed classes of GS (Table 7).

Fatty acid profiles in initial GS could be distinguished from those of experimental fish (Wilks' lambda: $F = 23.98$; df = 6, 14; $P < 0.001$) based on the abundances of two (14:0 and 14:1) of the 27 measured FAs (Table 4). The first canonical variate (Figure 1, lower panel) maximally separated initial fish FA profiles from those of the experimental fish and accounted for 96% of the variance. The second canonical variate only accounted for an additional 4% of the variance but was statistically significant ($P = 0.014$). However, when initial fish profiles were sequestered, CDA was unable to discriminate among experimental fish classes (i.e., Wilks' lambda was not significant; data not shown). The loading scores (Table 4) on Can1 for 14:0 (−0.46) and 14:1 (1.25) indicated that 14:0 pulled experimental fish profiles in the negative direction of the canonical plot (Figure 1, lower panel), while 14:1 pulled initial fish profiles in the positive direction. This was corroborated by the trends seen in these two FAs (Table 7); levels of 14:0 in initial GS (2.39%) were nearly twice those of fish fed 2×/week (1.38%) and triple those of GS fed 1×/month (0.77%). Levels of 14:1 were also much higher in initial GS (1.36%); however, among fed fish, levels of 14:1 showed the opposite trend from 14:0 and increased from 0.03% in fish that were fed 2×/week to 0.28% in fish that were fed 1×/month.

DISCUSSION

The results of our study indicate that winter feeding regimen may be important for minimizing weight loss in baitfish, depending upon the cultured species. In the present feeding trial for FHM, fish that were fed 2×/week had a positive percentage weight gain (2.95%), while fish that were fed 1×/week and 1×/month lost weight (−2.3% and −10.1%, respectively). These data are in agreement with Ludwig (1996), who reported that FHM fed 5 d/week at 0.1% or 2% BW had increases in final BW. All GS lost weight in the feeding trial regardless of feeding regimen, in contrast to a study by McNulty et al. (2000), who reported that GS lost weight when they were unfed but had positive weight gains when fed at 1% and 2% BW/d with air temperature greater than 7°C. Since it is necessary to overwinter fish in ponds in the southeastern USA, it is important to elucidate feeding strategies that will allow for maximal survival of fish and that will possibly obtain positive weight gain.

The data presented are the first from feeding trials to evaluate winter feeding regimens and to measure FA composition in either FHM or GS. Although several published studies have described the feeding of GS under conditions of summer water temperatures (Gatlin and Phillips 1988; Lochmann and Phillips 1995; Rowan and Stone 1995; Lochmann et al. 2009, 2010), there is only one published report with growth data from an outdoor winter feeding trial (McNulty et al. 2000). Similarly, there is one published report for FHM (Ludwig 1996); however, in that study, only two feeding regimens were used based upon BW, and no data were collected on FA composition of the fish (Ludwig 1996; McNulty et al. 2000).

Warmwater fish do not feed as aggressively in cool or cold months compared to warmer months of the year, given that the metabolism of poikilotherms is reduced as water temperatures decrease. Webster et al. (1994c) reported that Channel Catfish *Ictalurus punctatus* lost weight during the winter, and this was confirmed by subsequent published data (Kim and Lovell 1995; Nanninga et al. 2011); however, Webster et al. (1992) found a slight increase in weight of Channel Catfish during the winter. There appeared to be some benefit in feeding FHM 2×/week, as they did exhibit minimal weight gain; however, 1×/week and 1×/month feeding offered no increases in BW. Likewise, none of the three feeding regimens evaluated in the present study resulted in a weight increase for GS. Although fish of both species lost weight, this did not appear to affect survival, with the exception of GS that were fed 1×/month. Although the survival of GS fed 1×/month (65%) was not statistically different ($P = 0.088$) from the survival of fish subjected to the other two feeding regimens (80% and 88% for 1×/week and 2×/week, respectively) at our chosen significance level ($P \leq 0.05$), the

TABLE 7. Whole-body fatty acid composition (%) of initial and fed Golden Shiners that were held at 8°C for 12 weeks. Values are least-squares means of three replicates and the pooled SE (SE_p). Within a row, means with different letters are significantly different ($P \leq 0.05$). Treatments are defined in Table 2. Fatty acid abbreviations are defined in Table 3.

Fatty acid	Initial	2×/week	1×/week	1×/month	SE_p	$Pr > F$
14:0	2.39 z	1.38 y	1.13 yx	0.77 x	0.14	0.003
15:0	1.05 z	0.61 y	0.67 y	0.64 y	0.06	0.002
16:0	20.23	18.92	20.40	19.74	1.32	0.848
17:0	1.60	1.32	1.60	1.69	0.10	0.124
18:0	4.91 y	6.52 zy	7.35 z	7.77 z	0.47	0.005
19:0	0.84	0.86	1.09	1.42	0.24	0.367
20:0	0.36	0.42	0.59	0.27	0.21	0.830
21:0	1.68	1.11	1.58	0.67	0.61	0.600
Sum of saturates	31.43	29.85	32.81	31.28	1.24	0.445
14:1	1.36 z	0.03 y	0.08 y	0.28 y	0.08	<0.001
16:1	8.99 z	5.82 z	5.03 z	4.35 z	0.49	<0.001
18:1(n-7) (VAC)	20.27	18.15	16.01	13.06	1.77	0.081
18:1(n-9)	5.09	4.71	4.59	4.76	0.16	0.250
20:1	0.82	1.42	1.42	1.79	0.34	0.251
22:1	1.33	1.61	1.10	0.42	0.42	0.262
24:1	2.02	2.76	2.51	2.81	0.76	0.920
Sum of monoenes	39.89 z	34.49 zy	30.76 yx	27.45 x	1.52	0.003
18:2(n-6) (LA)	7.53 z	8.04 z	5.99 zy	3.97 y	0.81	0.015
20:2(n-6)	1.16 y	1.94 z	2.08 z	2.33 z	0.21	0.010
18:3(n-3) (ALA)	0.95	1.33	1.52	1.96	0.30	0.163
18:3(n-6) (GLA)	2.47 z	1.56 y	1.26 y	1.14 y	0.15	<0.001
20:3 (all)	0.92	1.88	1.47	1.77	0.27	0.077
20:4(n-6) (ARA)	2.58 x	4.84 y	6.04 zy	8.24 z	0.56	<0.001
20:5(n-3) (EPA)	1.73 y	2.31 zy	2.84 zy	3.28 y	0.27	0.015
22:2	0.90	0.39	0.71	0.54	0.54	0.908
22:3(n-3)	0.34	0.33	0.76	0.49	0.51	0.976
22:4(n-6)	1.00	0.95	1.44	1.62	0.67	0.959
22:5(n-3) (DPA)	1.52 y	2.45 zy	2.67 zy	3.43 z	0.41	0.036
22:6(n-3) (DHA)	3.89 y	7.26 z	7.32 z	10.10 z	0.54	<0.001
PUFAs	24.99 y	33.30 z	34.09 z	38.88 z	1.79	0.003
Σn-3	9.00 y	15.25 z	15.82 z	20.55 z	1.03	<0.001
Σn-6	15.64	17.72	17.51	17.84	1.41	0.671
n-6/n-3	1.79 z	1.17 zy	1.12 zy	0.88 y	0.14	0.014

numerical magnitude of the loss and borderline probability suggest that this feeding regimen should be avoided.

This is the first report of FA composition of FHM when fed at low water temperatures, and there were dramatic differences in FA composition among the three feeding regimens used during the present study. These differences indicate that when feed consumption and/or growth is minimal, FAs are used differently compared to those in fish that experience some weight gain during the winter. Both SFAs and monoenoic FAs decreased from initial levels over the duration of the feeding trial; however, fish that were fed 1×/week and 1 ×/month had a significant increase in 24:1 compared to fish that were fed 2×/week, indicating that fish not actively growing may selectively incorporate this FA into cell membranes to survive the winter (Farkas et al. 2001). However, if fish are growing, even minimally, normal metabolic processes occur, and 24:1 is not sequestered. The overall reduction in SFAs and monoenoic FAs may be due to their use as energy sources (Bell et al. 2003). Although it has been reported that oleic acid (18:1[n-9]) is used as an energy source during winter (Henderson and Sargent 1985; Roy et al. 2017), only fish that were fed 1×/week had a significant reduction in oleic acid from initial levels. Tocher et al. (1998) suggested that 18:1(n-9) can be utilized as a substrate for PUFA synthesis, which may explain why the level of 20:3 increased in fish belonging to each feeding treatment.

While FHM had decreased SFAs and monoenoic FAs, PUFA composition had significant increases in certain FAs, with the magnitude of increases dependent on feeding regimen. There were increases in 20:3, ARA (20:4[n-6]), EPA (20:5[n-3]), and DHA (22:6[n-3]) in fish irrespective of feeding regimen. Fathead Minnow that were fed 2×/week and 1×/week had increases in LA (18:2[n-6]), but fish that were fed 1×/month had reduced levels compared to initial fish. Overall, the more frequent the feeding, the less PUFAs were conserved, with PUFA levels for the feeding treatments as follows: 2×/week < 1×/week < 1×/month.

This is also the first published report to elucidate the FA composition of GS when fed during simulated winter (coldwater) conditions. Lochmann et al. (2007) identified the major FAs in eggs of GS and found high concentrations of 16:0 (25.5–28.1%), 16:1(n-7) (5.8–8.1%), 18:0 (6.5–7.3%), 18:1 (17.3–19.2%), LA (3.1–4.6%), ARA (4.3–6.4%), and DHA (13.9–16.5%). The FA data from the present study are in general agreement with the FA composition in GS eggs, with the exception that levels of DHA in the current study are much lower than those previously reported for eggs. This is not surprising, as eggs typically have higher concentrations of PUFAs, especially n-3 PUFAs (Fuller et al. 2017).

Golden Shiner FA composition exhibited slightly different trends than those in FHM. All GS lost weight during the feeding trial, indicating that the studied temperature (8°C) was not conducive for growth, which was evidenced by fish utilizing monoenoic FAs (as opposed to SFAs) for energy, while conserving PUFAs, especially DHA. Interestingly, there was a trend toward conserving (increasing) DHA as feeding frequency decreased; concomitantly, the percentage of LA decreased as the number of feedings decreased. This could imply that LA was used as an energy source, while DHA was conserved for use in cellular membranes and in neurological tissues during colder water temperatures.

Our findings that FHM and GS conserve PUFAs and have static or reduced SFA and monoenoic FA concentrations in response to winter (suboptimal) water temperatures is consistent with Skalli et al. (2006), who reported that polar lipids in cell membranes of European Bass *Dicentrarchus labrax* increased when fish were reared at 22°C compared to fish grown at 29°C. Likewise, Correa et al. (2015) observed maintenance or slight reductions in monoenoic FAs but dramatically higher PUFA levels in Nile Tilapia *Oreochromis niloticus* grown at 22°C compared to fish reared at 28°C. Conservation of PUFAs also has been reported in fasting fish even when water temperatures were optimal for growth of that species. In a study by Webster et al. (1994b), Channel Catfish that were fasted for 12 weeks had higher PUFA concentrations in muscle and liver than non fasted fish; SFAs and dienoic FAs were static, whereas monoenoic FAs decreased in fasted fish.

Phospholipids are a primary component of cellular membranes that optimize fluidity by incorporating PUFAs in the phospholipid molecules comprising the membranes (Webster and Lovell 1990; Webster et al. 1994a). As temperatures decrease, incorporation of PUFAs into cell membranes is vital to maintain the fluidity that is necessary for maintaining physical attributes and physiological functions. The results of the present study indicate that both FHM and GS sequester PUFAs at low water temperatures to maintain membrane fluidity, as has been observed for other species (Snyder and Hennessey 2003; Ng et al. 2015; Liqin et al. 2016; Liu et al. 2018).

Fish inhabiting cold climates are known to generally have higher levels of PUFAs in membranes compared to fish from warmer climates (Evans et al. 2012). Based on the current results, it appears that fish may conserve PUFAs during cold (suboptimal) water temperatures and that feeding frequency may also trigger PUFA sequestration, as both FHM and GS exhibited increases in PUFAs, especially DHA, when feeding frequency decreased. This is in contrast to the results of Bansemer et al. (2018), who reported that Yellowtail Jacks *Seriola lalandi* lost weight when fed at various frequencies and/or percentages of BW, but there was no trend in conservation of PUFAs under different feeding strategies when fish were fed at suboptimal temperatures. Further research is needed to more fully elucidate this aspect of fish feeding at cold (suboptimal) water temperatures.

Commercial baitfish farmers raising FHM may benefit from winter feeding 2×/week when weather conditions allow. Farmers do not feed FHM large amounts of commercial feed even in the summer, as this particular species relies heavily on the primary productivity of the pond for growth (Stone et al. 2016). However, natural food sources are not as plentiful during the winter. Fathead Minnow receiving feed 2×/week actually gained a modest amount of weight (3%) compared to weight losses of approximately 2% for the 1×/week treatment and 10% for the 1×/month treatment. Although the amount of weight gained in the 2×/week treatment was minimal, positive growth would be more advantageous to farmers than having their standing crop lose weight that must be regained in the spring, when water temperatures are higher and more conducive for growth. Interestingly, feeding 2×/week in similar experiments with centrarchids (Bluegill *Lepomis macrochirus*; and hybrid Bluegill [female Green Sunfish *L. cyanellus* × male Bluegill]) also raised in Arkansas led to substantial weight losses (12–20%) under all feeding regimens examined (2×/week, 1×/week, and 1×/month) at a low water temperature comparable to the one used in this study (Roy et al. 2017). Fathead Minnow in the 2×/week treatment also had a better K than those in the other two treatments (Table 2). Hence, FHM appear able to preserve robustness after long periods of exposure to

the temperature examined in this study if adequate feed rations are provided. However, further studies in commercial settings under fluctuating temperature conditions would provide more insight into the response of FHM to various winter feeding regimens.

Feeding GS 2×/week did not prove as advantageous as the same feeding regimen for FHM at the temperature examined. Golden Shiners lost approximately 7–10% of their BW at all feeding regimens, and there were no differences in weight loss. However, it is worth noting that GS in the 2×/week treatment had a significantly higher *K*-value than those in the other two feeding regimens (Table 6), suggesting that fish receiving feed 2×/week may display overall better condition following the winter months. It should be noted that in this laboratory, study temperature did not fluctuate as it would have in a commercial pond. However, previous work by researchers in Arkansas revealed that it was advantageous to feed GS at 1–2% BW when pond water temperatures exceeded 7.2°C (Rowan and Stone 1992, 1994, 1995). As in the case of FHM, further field studies are needed to refine practical feeding recommendations at the commercial level for GS.

In conclusion, FHM and GS generally lost weight when water temperatures reached 6–8°C; however, this did not appear to negatively affect survival. Furthermore, key PUFAs, especially DHA, were conserved for physiological needs in both species, while monoenoic FAs and some SFAs were utilized as energy sources.

ACKNOWLEDGMENTS

The Arkansas Department of Agriculture and the U.S. Department of Agriculture (USDA) Agricultural Research Service (Project Number 6028-31630-008-00D) both provided funds to support this research. Sonya Lockett provided assistance in procuring articles referenced in this paper; Sathyanand Kumaran supplied technical assistance; Rebecca Roberts provided assistance with FA analyses; and Paxton Harper and Chris Scheiderer provided technical support in sample preparation. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer. There is no conflict of interest declared in this article.

REFERENCES

Bansemer, M. S., D. A. J. Stone, T. D'Antignana, P. Skordas, L. Kuerschner, and K.-L. Currie. 2018. Optimizing feeding strategies for Yellowtail Jack at winter water temperatures. North American Journal of Aquaculture 80:128–140.

Bell, J. G., F. McGhee, P. J. Campbell, and J. R. Sargent. 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic Salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out." Aquaculture 218:515–528.

Correa, C. F., R. O. Nobrega, B. Mattion, J. M. Block, and D. M. Fracalossi. 2015. Dietary lipid sources affect the performance of Nile Tilapia at optimal and cold, suboptimal temperatures. Aquaculture Nutrition 23:1016–1026.

Evans, C. W., D. E. Williams, M. Vacchi, M. A. Bramble, and A. L. Devries. 2012. Metabolic and behavioural adaptations during early development of the Antarctic Silverfish, *Pleuragramma antarcticum*. Polar Biology 35:891–898.

Farkas, T., E. Fodor, K. Kitajka, and J. E. Halver. 2001. Response of fish membranes to environmental temperature. Aquaculture Research 32:645–655.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497–509.

Fuller, S. A., S. D. Rawles, M. E. McEntire, T. J. Bader, M. Riche, B. H. Beck, and C. D. Webster. 2017. White Bass (*Morone chrysops*) preferentially retain n-3 PUFA in ova when fed prepared diets with varying FA content. Lipids 52:823–836.

Gatlin, D. M., and H. F. Phillips. 1988. Effects of diet form on Golden Shiner *Notemigonus crysoleucas* performance. Journal of the World Aquaculture Society 19:47–50.

Hair, J. F., R. E. Anderson, R. L. Tatham, and W. C. Black. 2005. Multivariate data analysis, sixth edition. Prentice Hall, Englewood Cliffs, New Jersey.

Henderson, R. J., and J. R. Sargent. 1985. Chain-length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in livers of Rainbow Trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology 82B:79–85.

Hurst, T. P. 2007. Causes and consequences of winter mortality in fishes. Journal of Fish Biology 71:315–345.

Johnson, T. B., and D. O. Evans. 1990. Size-dependent winter mortality of young-of-the-year White Perch: climate warming and invasion of the Laurentian Great Lakes. Transactions of the American Fisheries Society 119:301–313.

Johnson, R. A., and D. W. Wichern. 2002. Applied multivariate statistical data analysis, 5th edition. Prentice Hall, Englewood Cliffs, New Jersey.

Kim, M. K., and R. T. Lovell. 1995. Effect of overwinter feeding regimen on body weight, body composition and resistance to *Edwardsiella ictaluri* in Channel Catfish, *Ictalurus punctatus*. Aquaculture 134:237–246.

Lemke, M. J., and S. H. Bowen. 1998. The nutritional value of organic detrital aggregate in the diet of Fathead Minnows. Freshwater Biology 39:447–453.

Liqin, J. L., K. Jiang, L. Mei, B. Wang, L. Han, M. Zhang, and W. Lei. 2016. Low temperature stress on the hematological parameters and HSP gene expression in the Turbot *Scophthalmus maximus*. Chinese Journal of Oceanology and Limnology 34:430–440.

Liu, C., Y. Zhou, K. Dong, D. Sun, Q. Gao, and S. Dong. 2018. Differences in fatty acid composition of gill and liver phospholipids between steelhead trout (*Oncorhynchus mykiss*) and Atlantic Salmon (*Salmo salar*) under declining temperatures. Aquaculture 495:815–822.

Lochmann, S. E., K. J. Goodwin, R. T. Lochmann, N. M. Stone, and T. Clement. 2007. Volume and lipid, fatty acid, and amino acid composition of Golden Shiner eggs during a spawning season. North American Journal of Aquaculture 69:116–126.

Lochmann, R., and H. Phillips. 1995. Comparison of rice bran oil, poultry fat, and cod liver oil as supplemental lipids in feeds for Channel Catfish and Golden Shiner. Journal of Applied Aquaculture 5:47–55.

Lochmann, R., and H. Phillips. 1996. Stable isotopic evaluation of the relative assimilation of natural and artificial foods by Golden Shiners *Notemigonus crysoleucas* in ponds. Journal of the World Aquaculture Society 27:168–177.

Lochmann, R., T. Sink, and H. Phillips. 2009. Effects of dietary lipid concentration, a dairy-yeast prebiotic, and fish and nonfish protein sources on growth, survival, and nonspecific immune response of Golden Shiners in indoor tanks and outdoor pools. North American Journal of Aquaculture 71:16–23.

Lochmann, R., T. D. Sink, H. Phillips, and R. Chen. 2010. Evaluation of a dietary dairy-yeast prebiotic in juvenile Golden Shiners in ponds. North American Journal of Aquaculture 72:164–171.

Ludwig, G. M. 1996. Seasonal growth and survival of rosy red and normal-colored Fathead Minnow receiving different feed rations. Progressive Fish-Culturist 58:160–166.

McNulty, E., and N. Stone. 1997. Winter feeding helps maintain baitfish pond blooms. Arkansas Aquafarming 15:4–5.

McNulty, E., N. Stone, and S. Lochmann. 2000. Effect of winter feeding rate, feed form and trigger temperature on yield and condition of Golden Shiners *Notemigonus crysoleucas*. Journal of Applied Aquaculture 10:69–82.

Miranda, L. E., and W. D. Hubbard. 1994. Length-dependent winter survival and lipid composition of age-0 Largemouth Bass in Bay Springs Reservoir, Mississippi. Transactions of the American Fisheries Society 123:80–87.

Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5:600–608.

Nanninga, A. S., C. R. Engle, N. Stone, and A. E. Goodwin. 2011. Effects of winter feeding of Channel Catfish on production in multi-batch systems. North American Journal of Aquaculture 73:60–67.

Ng, W. K., T. Sigholt, and G. Bell. 2015. The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic Salmon (*Salmo salar*) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquaculture Research 35:1228–1237.

Ott, R. L. 1977. An introduction to statistical methods and data analysis. Duxbury Press, North Scituate, Massachusetts.

Rowan, M., and N. Stone. 1992. Winter feeding of Golden Shiners. Arkansas Aquafarming 10:4–5.

Rowan, M., and N. Stone. 1994. Winter feeding of Golden Shiners: year II. Arkansas Aquafarming 12:5–6.

Rowan, M., and N. Stone. 1995. Effect of satiate or restricted feeding on Golden Shiner *Notemigonus crysoleucas* production in earthen ponds. Journal of the World Aquaculture Society 26:460–464.

Roy, L., A. Kelly, C. Engle, and N. Stone. 2013. Unusual fish losses observed by Arkansas baitfish/sportfish farmers during the winter of 2012/2013. Arkansas Aquafarming 30:4–6.

Roy, L. A., S. D. Rawles, A. M. Kelly, C. D. Webster, N. Stone, and A. Haukenes. 2017. Effect of winter feeding frequency on growth, survival, and fatty acid composition of juvenile Bluegill (*Lepomis macrochirus*) and hybrid Bluegill (*L. cyanellus* × *L. macrochirus*). Aquaculture 479:780–789.

Schultz, E. T., D. O. Conover, and A. Ehtisham. 1998. The dead of winter: size-dependent variation and genetic difference in seasonal mortality among Atlantic Silverside (Atherinidae: *Menidia menidia*) from different latitudes. Canadian Journal of Fisheries and Aquatic Sciences 55:1149–1157.

Skalli, A., J. H. Robin, N. Le Bayon, H. Le Delliou, and J. Person-Le Ruyet. 2006. Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European Sea Bass (*Dicentrarchus labrax*). Aquaculture 255:223–232.

Snyder, R. J., and T. M. Hennessey. 2003. Cold tolerance and homeoviscous adaptation in freshwater Alewives (*Alosa pseudoharengus*). Fish Physiology and Biochemistry 29:117–126.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science 60:1129–1157.

Stone, N. M., A. M. Kelly, and L. A. Roy. 2016. A fish of weedy waters: Golden Shiner biology and culture. Journal of the World Aquaculture Society 47:152–200.

Stone, N. M., E. Park, L. Dorman, and H. Thomforde. 1997. Baitfish culture in Arkansas: Golden Shiners, Goldfish, and Fathead Minnows. University of Arkansas, Cooperative Extension Program MP 386, Pine Bluff.

Stone, N. M., L. A. Roy, and E. D. Park. 2019. Fathead Minnow reproduction: implications for commercial culture. Journal of the World Aquaculture Society 50:267–298.

Tocher, D. R., M. J. Leaver, and P. A. Hodgson. 1998. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Progressive Lipid Research 37:73–117.

Toneys, M. L., and D. W. Coble. 1979. Size, related first winter mortality of freshwater fishes. Transactions of the American Fisheries Society 108:415–419.

Webster, C. D., and R. T. Lovell. 1990. Response of Striped Bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture 90:49–61.

Webster, C. D., R. T. Lovell, and J. A. Clawson. 1994a. Ratio of 20:3(n-9) to 20:5(n-3) in phospholipids as an indicator of dietary sufficiency in Striped Bass, *Morone saxatilis*, and palmetto bass, *M. saxatilis* × *M. chrysops*. Journal of Applied Aquaculture 4:75–90.

Webster, C. D., J. H. Tidwell, L. S. Goodgame, J. A. Clark, and D. H. Yancey. 1992. Winter feeding and growth of Channel Catfish fed diets containing varying percentages of distillers grains with solubles as a total replacement of fish meal. Journal of Applied Aquaculture 1:1–14.

Webster, C. D., J. H. Tidwell, L. S. Goodgame, and D. H. Yancey. 1994b. Effects of fasting on fatty acid composition of muscle, liver, and abdominal fat in Channel Catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 25:126–134.

Webster, C. D., W. A. Wurts, J. H. Tidwell, L. S. Goodgame, J. A. Clark, and D. H. Yancey. 1994c. Effects of stocking density on growth of juvenile Channel Catfish overwintered in ponds. Journal of Applied Aquaculture 4:79–87.